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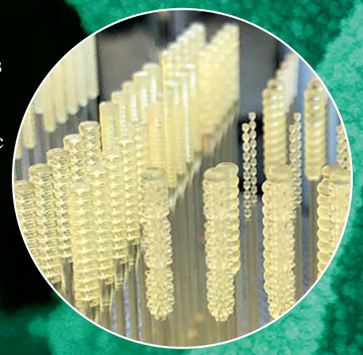
Tackling the COVID-19 Pandemic

To help the United States fight the COVID-19 pandemic, Lawrence Livermore did what it does best: draw on existing expertise and research activities to quickly bring together interdisciplinary teams and diverse technologies to address an urgent national challenge.

LONG before the COVID-19 pandemic gripped the globe, Lawrence Livermore National Laboratory had been preparing to help the nation contend with biological threats. From its inception, Livermore has focused its scientific and technological expertise on strengthening the security of the United States. Whatever the challenge involves—enhancing the nation's defense, reducing threats from terrorism, or addressing other issues of national importance—the Laboratory responds, creating teams from different disciplines and combining diverse technologies to find answers and solutions. The approach was no different when COVID-19 swept the nation in early 2020. Senior science advisor David Rakestraw, who coordinates the Laboratory's COVID-19 technical response, says, "Decades of mission-critical research plus current programmatic work conducted at the Laboratory have helped prepare us for this moment, such that we are at the forefront of many of the technologies relevant to addressing the challenges this pandemic presents."

In late January 2020, the Laboratory was alerted to the existence of a new, highly contagious novel coronavirus that



This image shows SARS-CoV-2 (round magenta objects) emerging from the surface of cells cultured in the lab. SARS-CoV-2, also known as 2019-nCoV, is the virus that causes COVID-19. (Image courtesy of NIAID.)

HPC Efforts Put All Hands on Deck

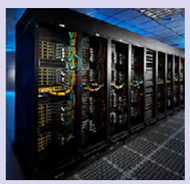
The Laboratory has long been involved in using supercomputing in the service of the nation and biological research applications. In 2013, the Laboratory established the Computational Predictive Biology initiative to address the evolution of diseases and biological threats and apply advanced high-performance computing (HPC) to biological research. The initiative became the foundation of a national collaboration—Biological Applications of Advanced Strategic Computing—of Department of Energy (DOE) laboratories, government agencies, academic institutions, and industry members.

Livermore's Deputy Associate Director for Computing Jim Brase says, "At first, we focused on simple approaches and pathways, but as system and facility capabilities grew, we started addressing the complexities of biology—efforts that required complicated computer models and enormous data sets**."** Three more collaborations formed to bring the power of HPC to human health challenges, such as cancer and infectious disease. A strategic partnership between Livermore and the American Heart Association creates computational tools to address cardiovascular disease and drug safety. Accelerating Therapeutics for Opportunities in Medicine, a consortium founded by DOE, the National Cancer Institute (NCI), and Lawrence Livermore, combines HPC, diverse biological data, and scientific expertise to accelerate discovery of cancer therapies. NCI also teamed up with Lawrence Livermore, Los Alamos, Argonne, and Oak Ridge national laboratories in a pilot program to integrate supercomputing into cancer treatments. (See *S&TR* November 2016, pp. 4–11.) The Laboratory's expertise in predictive biology is also a key element of its Biosecurity program, which focuses on detection, characterization, and mitigation to keep the world safe from ever-changing biological threats.

With the arrival of COVID-19, DOE formed the National Virtual Biotechnology Laboratory, a consortium of 17 national laboratories, including Lawrence Livermore, Los Alamos, Lawrence Berkeley, and Sandia. Supercomputing facilities at Livermore and throughout the complex are being used to address epidemiological modeling, manufacturing, molecular design for medical therapeutics, testing R&D, and viral fate and transport. In March 2020, the White House Office of Science and Technology, DOE, and IBM launched the COVID-19 HPC Consortium to provide COVID-19 researchers with access to the world's most powerful supercomputers including the Laboratory's newest supercomputer cluster, the 6-petaFLOP Ruby. The consortium currently supports nearly 100 projects around the globe.

Livermore senior science advisor David Rakestraw, who coordinates the Laboratory's COVID-19 technical response, notes, "Over the past seven years, we put a large focus on using the computational resources at the Laboratory to try to accelerate the timescales for developing a response to an emerging biological threat. We did so by using our extensive computational capabilities and developing partnerships with universities, drug companies, and tech companies. That effort put us in a position where we had the applicable tools and partnerships in place to help with the pandemic response."





Livermore's supercomputers Quartz, Lassen, and Corona (left to right) are among those leveraged in the fight against COVID-19.

was first detected in China. "We were very involved with the initial concern over this unusual virus and what it might mean if it were to spread more widely," says Rakestraw. The Laboratory was well-positioned to help, as its research areas span from the molecular level to the national-response level. When the SARS-CoV-2 novel coronavirus arrived on the nation's shores, the Laboratory had tools and people in place to begin delving into the threat and making the "unknown" known. The Laboratory's COVID-19 efforts focus on three critical science areas—medical equipment, detection, and medical countermeasures—bringing together teams to tackle a kaleidoscope of projects for each.

Equipping for the Pandemic

By March 2020, the U.S. Department of Energy (DOE) established a National Virtual Biotechnology Laboratory (NVBL) to bring all the relevant capabilities of its 17 national laboratories to bear on the science and technology challenges of the disease, such as supply chain issues of critical medical supplies and equipment. For Lawrence Livermore, one of the national laboratories tasked with addressing this challenge, the focus turned to ventilators, consumables such as testing kits, and masks and respirators.

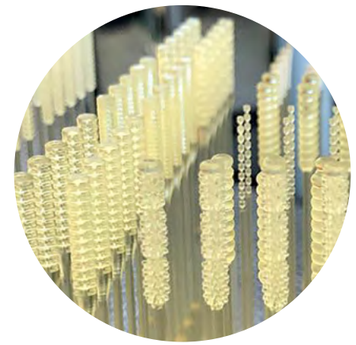
In the early phase of the pandemic, a nationwide shortage of ventilators loomed large as waves of critically ill people flooded hospitals in the Northeast United States. Immediately after the San Francisco Bay Area shelter-in-place order took effect in mid-March, a Livermore team headed by Jack Kotovsky formed with the goal of designing an easily assembled, durable mechanical ventilator using readily available parts not required by commercial ventilator manufacturers. Over long hours, the "skunk-works" team did most of their work—designing, prototyping, and testing—remotely, from home offices and garages. Within a week they had a conceptual design and

Early in the pandemic, a "skunk-works" team including (left to right) Ken Enstrom (seated), Greg Norton, and Aaron Sperry developed a production-ready ventilator built from components that do not impact the existing supply chain.

tried out components on a plywood "test stand." Two weeks later, an integrated system housed in a portable case was ready for full functionality testing. After five weeks, design, performance data, and accompanying documentation were submitted to the U.S. Food and Drug Administration (FDA) for emergency use authorization (EUA) approval. Just three months from the team's initial meetings, the Laboratory, working with industry partner BioMedInnovations, had created and tested an easily reproducible prototype and obtained EUA approval—a process that typically takes years. Kotovsky says, "When faced with global engineering challenges, like the ventilator shortage, it is hard to imagine a place better than Lawrence Livermore to find a solution. I am enormously proud of our team and grateful to the Laboratory for facilitating the work."

Around the same time, another Laboratory team formed to test prototype, nasal swabs manufactured via three-dimensional (3D) printing. When COVID-19 struck, only two companies—one in Italy and one in Maine—were manufacturing the swabs used to take samples of mucus from the nasal passages for testing. However, operations in the Italian company were severely impacted by the pandemic, and the Maine company was quickly overwhelmed with orders. Industry, academia, clinicians, and labs started a wide-scale grassroots effort to supply millions of 3D-printed COVID-19 test swabs.

Livermore's ad hoc, rapid response team of engineers including Angela Tooker, Eric Duoss, Maxim Shusteff, Razi Haque, Monica Moya, Dennis Freeman, Greg Larsen, Jack Davis, Du Nguyen, and Joshua DeOtte provided mechanical, sterilization, and other laboratory testing of hundreds of individual swabs based on more than a dozen novel designs from 3D-printing and biotech companies. Nearly six inches long, the swabs needed to be flexible enough to reach cells in the uppermost part of the throat without damaging tissue, but rigid enough to reach several inches into the nasal cavity and be rotated to collect samples. Tooker says, "We developed protocols and tested the 3D-printed swabs to provide quantitative data on swab performance, help narrow down design possibilities, and give clinicians confidence that the swabs were safe and effective to use." Mechanical tests conducted at Livermore's Advanced Manufacturing Laboratory simulated how the swabs might be used in a clinical setting.



Livermore researchers mechanically tested hundreds of 3D-printed nasal swabs based on more than a dozen designs developed through an informal consortium. The swabs pictured here were 3D-printed at Livermore from a biocompatible, surgical-grade resin and tested in Livermore's Advanced Manufacturing Laboratory.

Other Laboratory efforts to address equipment shortages included a partnership with industry and Oak Ridge National Laboratory to rapid-prototype 3D-printed vials for containing and transporting swabs used to collect samples and to examine shortages that could occur during vaccine distribution. Other efforts studied thermal methods for decontamination of N95 masks for reuse. "Along with the rest of the DOE complex, we keep analyzing the supply chain issues created by the pandemic with the goal of getting ahead of the curve and helping where and how we can," says Chris Spadaccini, division leader for Materials Engineering.

The Virus Hunt: Detection and Diagnosis

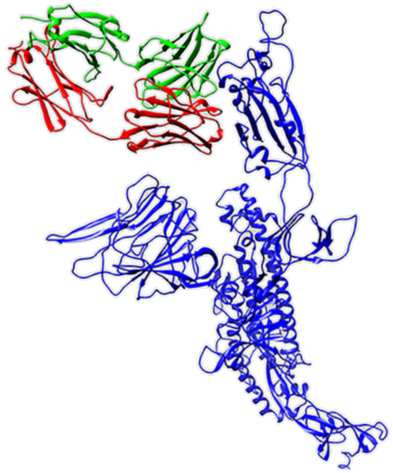
Livermore researchers support national efforts to detect the SARS-CoV-2 virus, developing rapid and accurate diagnostic technologies building on previous genomics work and existing research that supports the military. Biologist Crystal Jaing belongs to an NVBL working group tasked with developing new approaches for improved diagnostic testing. "One of the first projects we took on for the NVBL was to identify ways to extract viral RNA from nasal swabs that didn't use the chemical reagents employed by commercially available kits," says Jaing. In addition to Jaing, the Livermore team includes Jessica Wollard, Aubree Hinckley, James Thissen, Michael Morrison, and Nisha Mulakken.

Kit reagents break down the cells from the nasal swab samples to release DNA and RNA, so COVID-19 diagnostics tests can be performed. Most diagnostic tests use polymerase chain reaction (PCR),

a fast, highly sensitive, DNA-based technology, to identify the pathogens. The surge in demand for PCR-based COVID-19 tests had a rattle-down effect, leading to a shortage of reagents used in RNA extraction kits and a subsequent shortage of the kits themselves. Jaing and her team developed RNA extraction methods based on different reagents and tested them on archived COVID-19 samples provided by the California Department of Public Health (CDPH). The team compared their PCR results to results CDPH obtained from an FDA-approved SARS-CoV-2 test kit. Two of the Livermore-developed reagent formulations performed well and are now with CDPH, being validated on a larger sample size. Laboratory researchers also developed a rapid PCR diagnostic that showed promise for at-home use.

For another task, Livermore built on its decades of analysis of DNA and identification of pathogens, specifically by extending the capabilities of the Lawrence Livermore Microbial Detection Array (LLMDA). Developed at the Laboratory

Livermore's James Thissen (left) and Crystal Jaing (right) examine a microarray slide for the Lawrence Livermore Microbial Detection Array (LLMDA). In a single test, the LLMDA can detect up to 12,000 microbial species, including the COVID-19 virus and the viruses that cause co-infections, such as Influenza A, Human metapneumovirus, Human parvovirus, and Haemophilus influenzae.



A Livermore-designed machine-learning platform generated computer models showing the 3D structure of an antibody candidate (green and red) alongside the spike protein of SARS-CoV-2 (blue). The antibody, which binds with SARS-CoV-1 but not SARS-CoV-2, was the starting point for designing an antibody to the virus that causes COVID-19.

in 2008 by a team of biologists and bioinformatics specialists, the LLMDA simultaneously identifies up to 12,000 microbial species in a single test within 24 hours. (See *S&TR* April/May 2018, pp. 16-17.) In spring 2020, Jaing's team set out to design a COVID-19 "signature" that could be used by the LLMDA to detect the presence of the SARS-CoV-2 virus. Jaing explains, "First, we had to identify regions in the genome of the SARS-CoV-2 virus that were unique from other viruses, were conserved—that is, can be detected in the sequences of many variants—and were short in length." The SARS-CoV-2 genome has about 30,000 base pairs, a typical length for a coronavirus but long for viruses in general. For the array, the team had to identify regions about 60 bases long. Using the Laboratory's bioinformatics capabilities, the team analyzed more than 41,000 SARS-CoV-2 genomes in just a few months. They identified 78 conserved regions, designed signatures corresponding to those regions, and incorporated them into the array. "With this many signatures from conserved regions, we should still be able to detect and identify the virus as it mutates and evolves over time," says Jaing.

Jaing's team was also tasked with identifying additional pathogens in COVID-19 samples to ascertain what other infections were commonly found with the disease and whether there was a pattern. CDPH provided 200 samples taken from nasal and nose-and-throat swabs, evenly divided between those samples that had tested positive for COVID-19 and those that had not. After analyzing the samples with the LLMDA, the team found some of the COVID-19-positive samples also had co-infections, such as Influenza A and Streptococcus pneumoniae. "It will be interesting to compare our results to the patient data and see whether the more severe COVID-19 cases have more than one infection," says Jaing.

Designing Medical Countermeasures

Identifying that the virus is present is only the beginning. Fighting the virus requires medical countermeasures: vaccines to prevent or mitigate infection, and antibodies and antivirals to treat an infection. In the medical countermeasure arena, Livermore researchers combined artificial intelligence, machine learning, bioinformatics, and supercomputing to uncover candidates for new antibodies and antivirals that could fight the novel coronavirus. The Laboratory was well-positioned for the task, given its mission focus on biosecurity and use of high-performance supercomputing to support projects and partnerships to combat disease and aid human health. (See the box on p. 6.)

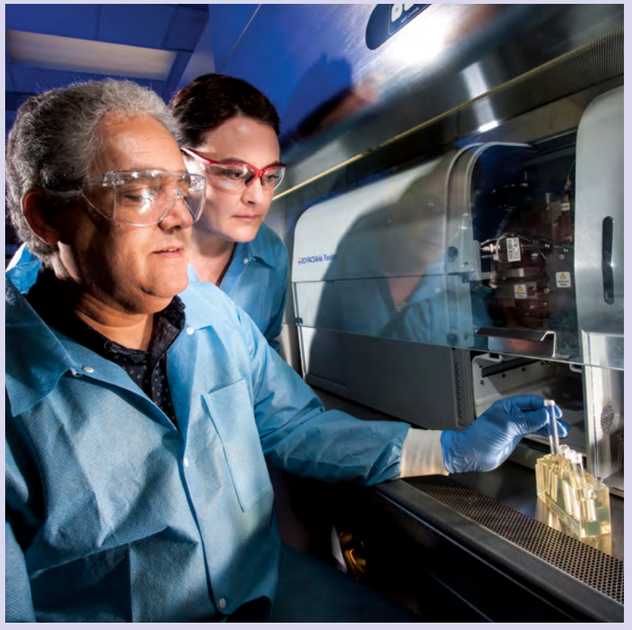
Initial work on sequencing the SARS-CoV-2 viral proteins began in late January 2020. Felice Lightstone, Livermore's technical lead for medical countermeasure R&D, notes, "The pandemic required the Laboratory to pivot quickly to meet this

Laboratory Directed Research and Development (LDRD) Program Invests in COVID-19 Research

Livermore's LDRD program invests in high-risk, potentially high-payoff research to meet some of the nation's most difficult challenges. As a key source of internally directed research funds, the program enabled the Laboratory to rapidly launch five projects related to COVID-19 early in 2020.

According to Doug Rotman, who leads the LDRD program at Livermore, "We had several scientists contact us with ideas regarding how they could help our Laboratory respond to the national COVID challenge, including emerging needs related to therapeutic targets and designs. Working closely with science leaders across the Laboratory, the LDRD program rigorously and quickly evaluated proposals and approved funding needed to start work." The LDRD-sponsored projects focus on leveraging Livermore's capabilities in high-performance computing, simulation, and data science—combined with Livermore's expertise in bioscience and bioengineering—to model, identify, and validate possible antiviral and antibody candidates. Computational scientists are also applying machine-learning tools to existing medical data sets to identify risk factors for COVID-19 patients.

In early 2021, the LDRD program funded research aimed at antibody screening technology to significantly reduce the time needed to develop countermeasures. The team includes experts in biophysics, bioengineering, synthetic biology, and molecular biology, who anticipate that their approach will enable researchers to rapidly identify and validate antibodies capable of neutralizing the virus.



Laboratory Directed Research and Development investments enabled Livermore scientists, including (left) biologist Matt Coleman and immunologist Amy Rasley, to explore how nanolipoprotein technology can be used to develop new vaccines. Laboratory biologists (top) Jessica Wollard and James Thissen use molecular-based technologies, such as polymerase chain reaction, microarray and genomic sequencing, to characterize microbes and pathogens in samples. (Above) Nick Fischer works with the LLMDA. (Photos by Randy Wong.)

national emergency." Lightstone points out that sequencing genomes for medical countermeasures research differs from traditional genome sequencing, requiring the creation of 3D models of the key viral proteins. In early February 2020, Livermore's Adam Zemla published a preliminary set of predictive 3D protein structures based on the genomic sequence of the SARS-CoV-2 virus and the known structure of a protein found in the original SARS virus. Starting with these preliminary protein models and a few antibodies known to bind and neutralize SARS-CoV-1, a team led by Daniel Faissol and Thomas Desautels used the Mammoth and Catalyst HPC clusters to screen for antibodies capable of binding to the SARS-CoV-2 spike protein.

The team employed a modeling platform that integrated experimental data, structural biology, bioinformatic modeling and molecular simulations—all driven by a machine-learning algorithm. The platform was used to identify potential high value modifications to the antibodies from the 2002 SARS virus so that the antibodies would bind and, therefore, neutralize SARS-CoV-2. By mid-March 2020, Lawrence Livermore researchers had identified about 20 promising antibody designs after simulating 90,000 antibodies chosen by the machine-learning model from a total of 10^{40} possible candidates. Although none of the 20 antibody designs turned out to bind strongly to the virus, subsequent design iterations yielded significantly improved candidates, several of which have been experimentally validated to neutralize SARS-CoV-2 while maintaining neutralization of SARS-CoV-1. The team is working with external partners to redesign existing antibody drug products to achieve binding and neutralization to SARS-CoV-2 escape variants of concern as well as beginning to work toward antigen design for a pan-coronavirus vaccine. 3D models of the SARS-CoV-2 proteins and the antibody

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structures are accessible through a public database, covid19drugscreen.llnl.gov.

In addition to groundbreaking work on antibodies, Livermore researchers focused attention on antivirals. Once infection sets in, antivirals—therapeutic drugs that treat the infection—can also be employed. "Antiviral drugs can sometimes be more

Livermore virologist Monica Borucki, along with other experimentalists from the Microbiology and Immunology Group, collaborated with Lawrence Livermore computational teams on projects focused on virtual antibody design and virtual screening of small molecule antivirals.

effective than therapeutic antibodies in treatment," explains Lightstone. Antiviral drugs frequently target the viral replication proteins, which are less likely to mutate. As a first step in developing new antiviral drug therapies to fight COVID-19 infection, a group led by Lightstone and Jonathan Allen used Livermore's Quartz, Corona, Lassen, and Ruby supercomputer clusters to screen small molecules against two COVID-19 proteins. Small molecules and drug therapies resulting from them need to be carefully targeted so that they only bind to viral proteins and not to similar proteins that exist in patients. Both physics-based and machine-learning methods were used to screen existing molecules. To suggest new compounds, a Livermore-developed machine-learning model was used to train the model on the 1.6 billion known molecules and 1 million additional compounds that looked promising for treating COVID-19. The model, which reduced training time from 24 hours to 23 minutes, was named a finalist for the prestigious Gordon Bell Special Prize for High Performance Computing-Based COVID-19 Research. The model is being added to the active learning loop that suggests novel molecules with improved properties and efficacy.

Once promising small molecules were identified, Livermore virologists and researchers, including virologist Monica Borucki, moved into the experimental phase of the task, investigating efficacy and safety in a laboratory setting. But one phase does not simply leave the other behind. "We iterate, folding the experimental results back into our calculations, to improve our predictions," says Lightstone. "The closer we make our predictions match the experimental data, the faster we can arrive at an effective drug therapy."

A Twist in the Plot

By their very nature, viruses mutate frequently, and the novel coronavirus is no exception. Most of these mutations

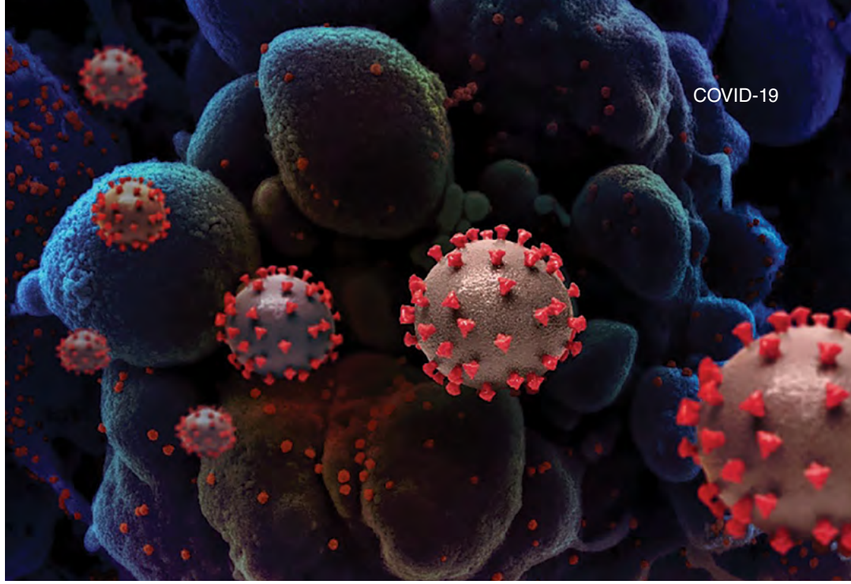
have been minor; however, in fall 2020, reports surfaced of a new variant with 23 mutations, some of which could enable COVID-19 to spread faster and easier than before. Borucki, who has studied other emerging pandemics including the Middle East Respiratory Syndrome and Zika virus, notes, "We expect new variants of the novel coronavirus will continue to emerge. As they do, we in the scientific community will need to deepen our understanding of the mutations and how they spread, adjust our detection technologies accordingly, and continue to develop countermeasures."

Borucki adds that the increased transmissibility of variants highlights the importance of fighting pandemic fatigue. "We must continue our efforts to stop viral transmission chains and continue to distribute vaccines to contain the pandemic," she says.

The Story Continues

The Laboratory's work on COVID-19 is far from over. In one ongoing effort that supports the COVID-19 research community, the Laboratory is creating a diagnostic-testing knowledge base

Environmental scientist Staci Kane collects a sample from a door on the Laboratory's main site for a research study to evaluate how environmental sampling might be used as an added tool in COVID-19 prevention and response efforts.



The RNA-based coronavirus responsible for COVID-19, SARS-CoV-2, is covered with a crown or "corona" of spike proteins. These proteins bind to ACE2, a protein found on the surfaces of many human cells, allowing cell and virus membranes to fuse, providing a pathway for the virus to enter the cell. The viral RNA then hijacks the cell's protein-making machinery to make new copies of the virus. The SARS-CoV-2 is about 80 nanometers in diameter, nearly 1,000 times smaller than the cells it infects.

that includes information regarding the microbes present in clinical samples obtained from COVID-19 patients, as well as genomic data regarding virus mutations. Even with effective vaccines in place, new tests are needed to monitor susceptibility, infection, and immunity. The Laboratory is also evaluating clinical and environmental tests that could help the Laboratory and other organizations determine how, when, and where to remove restrictions and open safely, without causing new infections.

"The COVID-19 pandemic has been one of the most significant challenges to the safety and security of our nation in the past century," says Rakestraw. "Here at Lawrence Livermore we were ready and able to quickly draw upon our deep technical expertise, ongoing research to improve the nation's biosecurity, and expansive infrastructure to offer help and solutions to a wide range of science and engineering issues." These contributions drew on specific capabilities developed in anticipation of a large-scale biological event and on the rapid application of broad capabilities to solve emerging challenges difficult to anticipate. "We are still living the story of the pandemic," says Rakestraw. "Whatever happens next, and then after that, the Laboratory is ready to help the nation face the future."

–Ann Parker

Key Words: Accelerating Therapeutics for Opportunities in Medicine, antibodies, antivirals, Biological Applications of Advanced Strategic Computing, biosecurity, Computational Predictive Biology initiative, COVID-19, detection, diagnosis, high-performance computing (HPC), Laboratory Directed Research and Development (LDRD), Lawrence Livermore Microbial Detection Array (LLMDA), medical countermeasures, medical equipment, nasal swabs, National Virtual Biotechnology Laboratory (NVBL), novel coronavirus pandemic, polymerase chain reaction (PCR), RNA, severe acute respiratory syndrome (SARS), SARS-CoV-2, small molecules, supply chain, vaccine, ventilator, virology.

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